**P-body Quantification Work Flow with ImageJ/Fiji**

1. Open Fiji Software, export data from Lif file
   1. Export individual channel from Lif file
      1. Plugins -> macros -> run
      2. Select file “Lif-Projector.jim
      3. Select folder in which Lif file resides (can’t be anything else in there)
      4. ! Select: close results images (if saved) !
   2. Will get folder – Zproj, where all Z projections are exported. All channels will have the same color, this is normal and will be changed later (check this)
2. Merging required images

(Color code for macros: C1=RED, C2=GREEN, C3=BLUE, C4=GREY, C5=CYAN, C6=MAGENTA, C7=YELLOW)

* 1. Make 3 empty folders for the channels that will be used, in this case: C=0 (DCP1-mTurquoise), C=2 (VCS-mScarlet), C=3 (magenta, chloroplasts)
  2. Search for channels in the ZProj folder and copy the images in the correct folder (search explorer in file using: “C=0” (with speech marks), and simply select all and copy into the respective folder) --- If experiment settings are different, need to change the colors in the macros (list1/2/3 etc) at the bottom to correspond to color code listed above
     1. Plugins -> macros -> run
     2. Select file “Automerge3.jim
     3. Select the folders for each channel when prompted (see header), make new folder called “Merge”

1. Training: Optional, already done for this type of experiment. If the images are very different, may need to redo
   1. Choose 3 representative images per treatment from merge folder
   2. Drag and drop into Fiji
      1. Image -> stacks -> image to stack
      2. Stacks -> make montage (scale factor: 1)
   3. Save as labeling.tif
   4. In search bar (in fiji), look for labkit -> open current image with labkit
   5. On the left, add labels for proteins eg. Background (0), VCS (1), DCP1 (2), both (3)
   6. Mark all (or as many as possible, and as precisely as possible) P-bodies according to the categories including background
      1. Labelling -> save labelling when finished (quick save in between to not lose progress)
      2. segmentation -> train classifier
   7. check the images:
      1. segmentation -> show result in imagej
      2. image -> adjust -> threshold (by using the slider, you can see what foci are being put into what category, eg: 0,0 will show only background, 1,1 will show all foci the programme thinks are VCS etc
   8. SAVE classifier when finished
2. Using Classifier to count P-bodies
   1. Open any image in Fiji and then with the labkit plugin
      1. Segmentation -> open classifier
      2. Others -> batch segment images
      3. Specify input directory “merge” and output directory as “Segment”
   2. Move back to Fiji
      1. Process -> batch -> macros
   3. Select segment folder as input directory and the corresponding fluorophore analysis folder as output eg: Analysis – CFP etc
   4. In the macros field, paste the correct macros. There are 3 for mTurqouise/CFP, mScarlet/RFP and both. The difference in these are the numbers after “Threshold(x,x)” ! These must match the order of the marking in labkit! eg in this case 1,1 is DCP1-CFP because DCP1-CFP is the first category after background
   5. Run the macros, copy the result into excel for further analysis

Misc: Adjust brightness

Process, batch macros, use “Adjust\_macros”. Can change the parameter by first determining desired minimum and maximum value for the images and replacing the values in the macros

Specify input and output folders and press run. To make other macros, use record function and carry out the steps in Fiji, they will be listed.

! Channels were labelled differently (C=x), than in the training